CONTAMINANT OF MALIC ACID: A MALIC ENZYME INHIBITOR

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A sample of malic acid (Mann Laboratories, C.P. grade, Lot #S1090) strongly inhibited the malic enzyme activity of a pig adipose tissue supernatant fraction (100,000 *g*), as measured by the method of Ochoa [1]. Thin-layer cellulose chromatography (Brinkmann MN-Polygram Cel-300-Ecteola) using diethyl ether–formic acid–H_2O (7:2:1) as mobile phase, showed a second slower-moving component in the malic acid sample. Other samples of malate that were not inhibitory to the enzyme ran as single spots in the same system.

Milligram quantitites of the contaminant were prepared by solvent elution on a silica gel column according to a procedure developed by Ichiyama [2] for quantitative resolution of intermediates of the citric acid cycle and gluconeogenic pathway. Fourteen grams of silicic acid (100 mesh powder) were mixed with 10.3 ml of 0.5 N H_2SO_4 in a mortar, added to a chromatographic tube (0.8 X 60 cm) with sufficient chloroform, and packed with a glass rod to 53 cm. The sample (134 mg of contaminated malate) was dissolved in 1.5 ml of 0.5 N H_2SO_4 and mixed with about 2 g of silicid acid. It was then layered on the column with 10 ml of chloroform. Elution was carried out successively with 60 ml of 0%, 280 ml of 20% and 700 ml of 30% butanol in chloroform, saturated with 0.5 N H_2SO_4. Individual fractions were titrated to pH 9 with 0.1 N KOH after addition of 4 ml of water. The contaminant was eluted in the 20%, and malate in the 30% butanol fraction (fig. 1). Inhibitory activity was found only in the contaminant peak which made up 13% of the total sample as measured by KOH titration.

Preliminary tests showed the inhibitor contained no keto or aldehyde groups, as determined by a negative dinitrophenylhydrazine reaction. The *p*-bromophenacyl ester prepared by the method of Shriner et al. [3], decomposed at approximately 230°, characteristic of an oxalic acid derivative. A specific test for oxalic acid using diphenylamine blue [4] was positive. As a final identification step, ^{14}C-labelled oxalate was run with the contaminated malate in the column chromatography system described above. The elution profile of the radioactivity coincided exactly with the inhibitor peak (fig. 1).

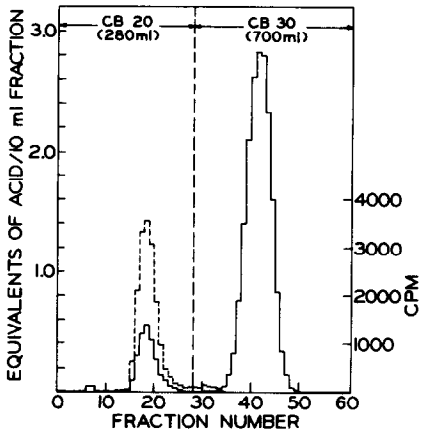

Fig. 1. Separation of contaminant from malic acid by silica gel column chromatography, in the presence of ^{14}C-oxalic acid. Conditions are given in the text.

From this evidence it was concluded that the second component in the malic acid sample was oxalic acid. Preliminary kinetic studies with the isolated inhibitor showed half-maximal inhibition of pig adipose tissue malic enzyme at 0.1 mM, in presence of 0.25 mM malate. However, a previous report [5] indicated that oxalate inhibition of pigeon liver malic enzyme was considerably less (half maximal at 1 mM, in

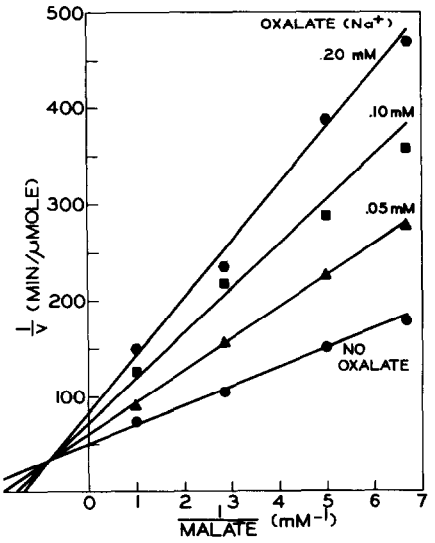

Fig. 2. Oxalate inhibition of pig adipose tissue malic enzyme. Assay system contained 0.1 mM NADP, 2.0 mM $MgCl_2$, 50 mM tris HCl (pH 7.4) and variable amounts of malate and oxalate. Rates of reduction of NADP were followed at 340 nm. Approximately .50 μg of pig adipose tissue protein were present. Temperature was 23°.

presence of 0.1 mM malate). Further kinetic studies were done with the pig adipose enzyme, and oxalate was found to be at least ten-fold more effective as a non-competitive inhibitor than the literature indicated (fig. 2). In addition, it was observed that high levels of Mn^{2+} (greater than 20 mM) almost completely eliminated the inhibition by 0.2 mM oxalate. Since the assay system reported by Strickland [5] contained 20 mM $MnCl_2$, it is probable that the lower potency of oxalic acid observed with pigeon liver malic enzyme is merely a reflection of unfavorable assay conditions, rather than an innate difference between the two enzymes.

A second labelled component has been observed in commercial ^{14}C-malic acid in this laboratory [6]. Chromatographic evidence suggests that it may also be oxalic acid. Other literature reports have invoked a possible interaction between D and L malate to account for a chromatographically distinct component of malate [7]. In view of the present findings, it seems more likely that oxalic acid is a relatively common contaminant of less pure grades of malic acid.

Acknowledgements

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